



# High time-resolution simulation of *E. coli* on hands reveals large variation in microbial exposures amongst Vietnamese farmers using human excreta for agriculture

Timothy R. Julian<sup>a,b,c,\*</sup>, Hasitha S.K. Vithanage<sup>d,e</sup>, Min Li Chua<sup>f</sup>, Matasaka Kuroda<sup>g</sup>, Ana K. Pitol<sup>a,h</sup>, Pham Hong Lien Nguyen<sup>i</sup>, Robert A. Canales<sup>j</sup>, Shigeo Fujii<sup>f</sup>, Hidenori Harada<sup>f,k,\*\*</sup>

<sup>a</sup> Eawag, Swiss Federal Institute of Aquatic Science and Technology, Überlandstrasse 133, CH-8600 Dübendorf, Switzerland

<sup>b</sup> Swiss Tropical and Public Health Institute, Socinstrasse 57, P.O. Box, CH-4002 Basel, Switzerland

<sup>c</sup> University of Basel, P.O. Box, CH-4003 Basel, Switzerland

<sup>d</sup> UNESCO-IHE Institute for Water Education, 2601 Delft, The Netherlands

<sup>e</sup> Sri Lanka National Water Supply and Drainage Board, Dehiwala-Mount Lavinia, Sri Lanka.

<sup>f</sup> Graduate School of Global Environmental Studies, Kyoto University, Yoshida-honmachi, Sakyo-ku 606-8501, Kyoto, Japan

<sup>g</sup> Graduate School of Engineering, Katsura Campus, Kyoto University, Nisikyo-ku, 615-8510, Kyoto, Japan

<sup>h</sup> Laboratory of Environmental Chemistry, School of Architecture, Civil, and Environmental Engineering (ENAC), École Polytechnique Fédérale de Lausanne (EPFL), CH 1015 Lausanne, Switzerland

<sup>i</sup> School of Environmental Science and Technology, Hanoi University of Science and Technology, Viet Nam

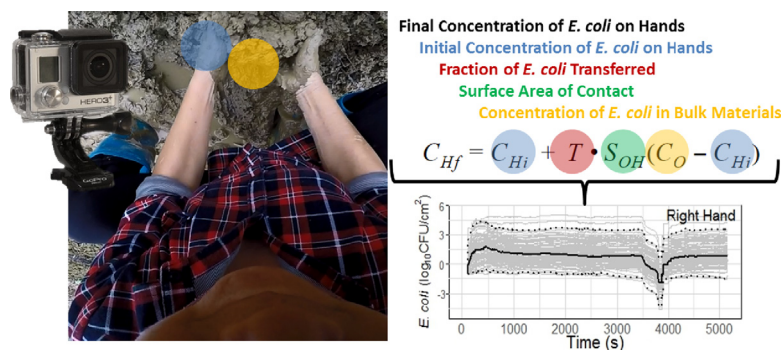
<sup>j</sup> Community, Environment & Policy Department, Mel and Enid Zuckerman College of Public Health, The University of Arizona, 1295 N. Martin Avenue, Campus PO Box 245210, Drachman Hall A229, Tucson, AZ 85724, USA

<sup>k</sup> Research Institute for Humanity and Nature, 457-4 Kamigamo Motoyama, Kita-ku 603-8047, Kyoto, Japan

## HIGHLIGHTS

- Videography and *E. coli* data are collected for farmers using human excreta.
- Data are used to simulate *E. coli* concentrations on hands over time.
- Farmers' left and right hands contacted a mean 360 and 401 objects per hour.
- Microbial exposures vary substantially between farmers.
- *E. coli* in excreta and on tools, and hand-to-mouth contact frequency, most impacted risk.

## GRAPHICAL ABSTRACT



## ARTICLE INFO

### Article history:

Received 30 January 2018

Received in revised form 6 April 2018

Accepted 6 April 2018

Available online xxxx

Editor: Simon Pollard

## ABSTRACT

Infectious disease transmission is frequently mediated by the environment, where people's movements through and interactions with the environment dictate risks of infection and/or illness. Capturing these interactions, and quantifying their importance, offers important insights into effective interventions. In this study, we capture high time-resolution activity data for twenty-five Vietnamese farmers during collection and land application of human excreta for agriculture. Although human excreta use improves productivity, the use increases risks of enteric infections for both farmers and end users. In our study, the activity data are integrated with environmental microbial sampling data into a stochastic-mechanistic simulation of *E. coli* contamination on hands and *E. coli* ingested. Results from the study include frequent and variable contact rates for farmers' hands (from 34 to

\* Correspondence to: T.R. Julian, Eawag, Swiss Federal Research Institute of Aquatic Science and Technology, Überlandstrasse 133, CH-8600 Dübendorf, Switzerland.

\*\* Correspondence to: H. Harada, Graduate School of Global Environmental Studies, Kyoto University, Yoshida-honmachi, Sakyo-ku, 606-8501, Kyoto, Japan.

E-mail addresses: [tim.julian@eawag.ch](mailto:tim.julian@eawag.ch), (T.R. Julian), [harada.hidenori.8v@kyoto-u.ac.jp](mailto:harada.hidenori.8v@kyoto-u.ac.jp), (H. Harada).

**Keywords:**

Quantitative microbial risk assessment  
Human excreta  
Land application  
Microlevel activity time series

1344 objects contacted per hour per hand), including highly variable hand-to-mouth contact rates (from 0 to 9 contacts per hour per hand). The frequency of hand-to-mouth contacts was substantially lower than the widely-used frequency previously reported for U.S. Office Workers. Environmental microbial contamination data highlighted ubiquitous *E. coli* contamination in the environment, including excreta, hands, toilet pit, hand-held tools, soils, surfaces, and water. Results from the simulation suggest dynamic changes in *E. coli* contamination on hands, and wide variation in hand contamination and *E. coli* ingested amongst the farmers studied. Sensitivity analysis suggests that *E. coli* contamination on hands and ingested doses are most influenced by contamination of handheld tools, excreta, and the toilet pit as well as by frequency of hand-to-mouth contacts. The study findings are especially relevant given the context: no farmers reported adequate storage time of human excreta, and personal protective mask availability did not prevent hand-to-mouth contacts. Integrating high time-resolution activity data into exposure assessments highlights variation in exposures amongst farmers, and offers greater insight into effective interventions and their potential impacts.

© 2018 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

## 1. Introduction

Transmission of many infectious diseases, such as enteric diseases, is mediated by human-environment interactions. People's movement through, and contact with, the environment contributes to fate, transport, and transmission of infectious diseases. Quantitative microbial risk assessment (QMRA) is one example of a framework used to integrate human-environment interaction data to quantify risks associated with specific activities, understand relative contributions of various exposure pathways, and identify effective infection control strategies (Haas et al., 2014; WHO, 2016). Within this framework, exposure assessment is the process of estimating magnitude and frequency of people's exposures to pathogens (Haas et al., 2014; WHO, 2016). The assessment is based on pathogen contamination estimates, treatment and/or intervention efficacy, and human-environment interaction data (WHO, 2016). Examples of human-environment interaction data include estimates of the amount of water and/or food ingested (intentionally or unintentionally) and frequency of hand-to-mouth contacts (Amha et al., 2015; Fuhrmann et al., 2016; Mattioli et al., 2015).

Exposure assessments, and in particular human-environment interaction data, are often based on simplified assumptions. For example, interaction data estimates (i.e., water, food, and soil intake; hand-to-mouth frequency) may be based on expert opinion, estimates from studies conducted in different contexts or study populations, or described as simplistic, linear, series of events (Amha et al., 2015; Genthe et al., 1999; Mattioli et al., 2015; Schöningh et al., 2007). Increasingly, research is recognizing the need for improved human-environment interaction data (i.e., drinking water consumption, wastewater contacts), leading to more robust, evidence-based, exposure and risk estimates (Gretsch et al., 2016; Kwong et al., 2016; Teunis et al., 2016).

One approach to improving human-environment interaction data is the collection of high time-resolution (per second) activity data via videography and video translation (Ferguson et al., 2006; Julian et al., n.d.; Zartarian et al., 1995). In brief, study participants are observed using videography, and translators (aided by software) convert the video into a detailed (second-by-second) time series of the study participants' environmental interactions. The resulting data is referred to as microlevel activity time series, or MLATS. The method can be used to estimate dermal, inhalation, non-dietary ingestion, and/or dietary ingestion exposures, as shown in chemical risk assessments (Beamer et al., 2009; Ferguson et al., 2013, 2006). The method has also been applied to microbial risks, primarily in the context of non-dietary ingestion exposures (Beamer et al., 2015; Julian et al., 2009; Julian and Pickering, 2015).

Here, we apply MLATS to model farmers' risks from use of human excreta in agriculture in Vietnam. Human excreta is used extensively for agriculture and aquaculture in Southeast Asia, and Vietnam in particular (Do et al., 2007; Giang et al., 2015). Use of excreta is driven in part by financial benefits of nutrient recovery. Excreta use offsets fertilizer

purchases and improves crop yield (Jensen et al., 2010). Use of human excreta in agriculture is also globally beneficial, as it captures and recycles nutrients. Nutrient capture diverts environmental pollution and offsets reliance on finite resources like phosphate rock (Cordell et al., 2011; Fuhrmeister et al., 2015; Heinonen-Tanski et al., 2005; Langergraber and Muellegger, 2005).

Despite the benefits, human excreta poses a health risk for farmers and end consumers. Excreta contains high concentrations of enteric pathogens including diarrheagenic *Escherichia coli*, *Salmonella* spp., rotavirus, norovirus, and *Campylobacter* spp.; hepatitis (hepatitis A and E); poliovirus; *Toxoplasma gondii*; and parasitic worms (Lam et al., 2015). Previous studies have identified increased risks of diarrheal disease generally, and helminth, hookworm, and *Trichuris Trichuria* infections specifically, for farmers reliant on human excreta (Blumenthal and Peasey, 2002; Do et al., 2007; Pham-Duc et al., 2014; Pham Duc et al., 2011).

Recommendations for reducing health risks from human excreta use are rarely met. Examples include storage of excreta for at least 6 months prior to use to allow sufficient time for inactivation of pathogens; incorporating additives (kitchen ash, waste, and/or lime) to reduce moisture, smell, increase pH, and combat flies; and using personal protective equipment (PPE), (masks, gloves, and boots) to reduce farmers' exposures to excreta (Mackie Jensen et al., 2008; Phuc et al., 2006; Winblad, 2004). In Vietnam, excreta is rarely stored for the recommended 6 months (Jensen et al., 2010; Mackie Jensen et al., 2008). One reason is the misalignment between recommended storage times and seasonal timing or frequency of required land application (Jensen et al., 2010; Mackie Jensen et al., 2008). Personal protective equipment, although perceived to be beneficial, is also often neglected due to costs and/or perceived convenience (Knudsen et al., 2008). Another contributing factor is the prevailing belief that smell is indicative of health risks; once smell has dissipated, concerns over health decline (Knudsen et al., 2008). Therefore, recommended infection control precautions should be optimized to decrease pathogen exposures while accounting for farmers' logistical, cultural, and behavioral concerns (Mackie Jensen et al., 2008).

In this study, we evaluate the use of high time-resolution activity data to estimate farmers' exposures to the fecal bacteria *E. coli* while using human excreta in Hanoi, Vietnam. Videography is used to capture detailed high time-resolution data, which is used as the basis for a stochastic-mechanistic simulation. The simulation is parameterized using both primary data collection (measured *E. coli* contamination on hands, surfaces, water, and excreta) and previously published data (transfer efficiency of pathogens, surface area of contacts). Results obtained from the stochastic-mechanistic exposure simulation are compared to more traditional approaches to estimate exposure. The comparison is used to evaluate the use of high time-resolution human-environment interaction data. Finally, the simulation is used to identify factors that contribute to increased exposure, and to reaffirm infection control practices that can reduce farmers' exposures and risks.

## 2. Materials and methods

### 2.1. Ethics

The following protocol was approved by the Ethics Commission of the Swiss Federal Institute of Technology in Zurich. Twenty-five (25) farmers were contacted through local village chiefs based on who was performing relevant agricultural activities (human excreta collection, transport, and/or land application). The farmers were provided with specific information about the research and research protocol. At the end of data collection, the farmers were also provided with general instructions on ameliorating risks from contact with human excreta. All information was provided in Vietnamese (translated from English) and was accompanied by a verbal explanation by a native Vietnamese speaker. Research proceeded only after written consent was obtained.

### 2.2. Study site

The study was conducted from November 2015 until February 2016 in Trai Hamlet, Van Tu commune, Phu Xuyen district in Hanoi, Vietnam. Trai Hamlet is a farming village with >240 households that relies heavily on human and animal excreta for agriculture (Giang et al., 2012). The primary occupation for most households is domestic-scale agriculture, including animal farming. Of the village's 56 ha land area, >90% is devoted to paddy fields and fish ponds (Giang et al., 2012). More than half (56%) of the households use dry toilets for sanitation, and most (93%) of these households use the human excreta for agriculture (Giang et al., 2012). The study dates were chosen to coincide with the seasonal cultivation of major crops within the region, including beans, rice, and other vegetables.

### 2.3. Cohort

In total, 25 farmers were enrolled in the study for activity data collection (i.e., videography): 11 farmers collected human excreta from their latrine, 12 applied excreta to land, and 2 both collected and applied excreta. Of these, 15 (60% of the 25 total) also provided hand rinse samples for enumerating *E. coli* contamination of hands. The vast majority (96%) of farmers were female, and the median [min, max] age was 56 [38, 64].

### 2.4. Simulation framework

An exposure assessment simulation was developed that estimates time series of *E. coli* contamination on the hands and *E. coli* ingested due to hand-to-mouth contact events. The simulation framework is mechanistic and incorporates stochasticity of model parameters to capture variability and uncertainty, following a previously established model for estimating microbial transfer between hands and objects (Julian and Pickering, 2015). In brief, the model simulates *E. coli* concentrations on hands by tracking the transfer of *E. coli* between hands in the environment. The time series of sequential contact events (termed microlevel activity time series, or MLATS) responsible for transfer is determined using videography and videotranslation (see Activity Data) (Julian et al., n.d.; Zartarian et al., 1995). We assume each contact between an object and a hand transfers bacteria to or from the hands based on: 1) bacteria contamination on the hands, 2) bacteria contamination on the object, 3) material properties of the object, and 4) the area of the hands involved in hand-to-object contact. Transfer for each contact event is modeled assuming bacterial transfer is a function of the difference (or gradient) in bacterial concentration between the hand and the object (Julian et al., 2009; Julian and Pickering, 2015). For surfaces, transfer was modeled as:

$$C_{Hf} = C_{Hi} + T \cdot S_{OH} (C_O - C_{Hi}) \quad (1)$$

where  $C_{Hf}$  is the final bacterial concentration on the hands (CFU/cm<sup>2</sup>),  $C_{Hi}$  is the initial concentration on the hands (CFU/cm<sup>2</sup>),  $T$ , transfer efficiency, is the proportion of bacteria that transfers between the object and the hands (unitless or g/cm<sup>2</sup>),  $S_{OH}$  is the fractional surface area of the hand in contact with the object, and  $C_O$  is the concentration on the object (CFU/cm<sup>2</sup> or CFU/g).

For bulk materials (ash, excreta, mud) and water, transfer is modeled as:

$$C_{Hf} = C_{Hi} + S_{OH} (T_B C_B - C_{Hi}) \quad (2)$$

where  $T_B$  is the transfer efficiency of bulk materials or water to hands (g/cm<sup>2</sup> or ml/cm<sup>2</sup>), and  $C_B$  is the concentration of the bulk material or water (CFU/g or CFU/ml).

Dose of *E. coli* ingested by the farmers is assumed to occur during hand-to-mouth contacts. Although object-to-mouth contacts may also increase dose, none were observed (see Activity Data). Dose is modeled using:

$$Dose = T_{HM} \cdot S_{HM} \cdot C_{Hf} \quad (3)$$

where  $T_{HM}$  is the proportion of bacteria that transfers between the hands and the mouth (unitless),  $S_{HM}$  is the surface area of the hand in contact with the mouth (cm<sup>2</sup>), and  $C_{Hf}$  is the bacterial concentration on the hands (CFU/cm<sup>2</sup>).

The simulation includes stochastic parameters, relying on Monte Carlo methods to incorporate variability and uncertainty. Parameter values are chosen from distributions which reflect variability and uncertainty for each parameter. Specifically, *E. coli* contamination of objects is randomized at the start of each simulation, whereas both transfer efficiency and surface area of the contact are randomized prior to each contact event. To instantiate the simulation, initial ( $t = 0$ ) *E. coli* concentration on hands are assumed to be 0.01 CFU *E. coli*/cm<sup>2</sup>, informed by *E. coli* measurements obtained at the start of videography (see Microbial contamination).

The primary outcomes from the simulation are the time series of *E. coli* concentration on the hands and ingested dose, defined here as the quantity of *E. coli* transferred to the mouth from hand-to-mouth contacts.

### 2.5. Parameter estimation

#### 2.5.1. Activity data

Activity data were collected following the method of Julian and Pickering (2015). In brief, video cameras on head straps (GoPro Digital Hero 4, Woodman Labs, San Mateo, CA) were worn by farmers. Video – in first person perspective – of the range of motion of the farmers' hands and the lower portion of their face was captured for the duration of their activity. The farmers were instructed to perform their typical activities as they usually would. Between 26 and 344 min of data were collected for each farmer.

The videos were translated using Virtual Timing Device Software for the Personal Computer (VTDPC, University of Arizona), which was based on the Virtual Timing Device Software (SamaSama Consulting, Sunnyvale, CA) (Julian et al., n.d.; Zartarian et al., 1995). Translation was performed by two researchers (co-authors HSKV and MLC) as previously described (Julian and Pickering, 2015; Zartarian et al., 1995). Both researchers were trained on the use of the software, with results from initial 10 min segment validated by a third researcher (AKP). For comparison, 30 min of the data were translated by both researchers (see Activity data and Supporting information, Comparison of Translators).

#### 2.5.2. Microbial contamination

Microbial contamination was indicated by the fecal bacteria *E. coli* because it is a commonly used fecal indicator bacteria, and there are

multiple, reliable, and field-portable measurement methods. *E. coli* contamination was measured on surfaces, in bulk materials, in water, and on hands following methods specified in the Supplemental information (Supplemental information, Methods). Although *E. coli* is not necessarily pathogenic, pathogenic strains (enteropathogenic, enterotoxigenic, and enterohemorrhagic) are amongst the leading causes of diarrheal disease (Kotloff et al., 2013). *E. coli* are also expected to be present in high concentrations in surfaces, bulk materials, and water in environments reliant on human excreta for agriculture.

**2.5.2.1. Probability distribution functions.** *E. coli* measurements from surfaces, bulk materials, and water were fit to probability distribution functions, defined for each object category. Probability distribution function parameters were estimated using the *fitdistr* package in R (R Core Team, 2016). When *E. coli* were detectable on 40% or more of a sample category, a normal distribution was fit to the log-transformed *E. coli* concentrations, with data below and above the limits of detection treated as left and right censored data, respectively. When <40% of samples had detectable *E. coli* contamination, the data were input directly into the simulation and resampled with replacement, with data below the limits of detection assumed to be uncontaminated (0 CFU/g or cm<sup>2</sup>).

### 2.5.3. Transfer

Transfer of *E. coli*, defined by the proportion transferred between the hands and objects on contact, was estimated based on a literature review (Table 1). Transfer (%) between surfaces and hands was assumed to be normally distributed, based on previous estimates of transfer rates (Julian et al., 2009; Lopez et al., 2013). Mean and standard deviation estimates are based on previously published experimental work using *E. coli*, *Acinetobacter baumannii*, or *Serratia rubidea* (Greene et al., 2015; Lopez et al., 2013).

Limited data were available to estimate transfer of *E. coli* between most bulk materials (ash, excreta) and hands, and between water and hands. Therefore, *E. coli* transfer was modeled based on the mass or volume of the bulk material transferred to the hands after contact and the concentration of *E. coli* in the material (Table 1) (U.S. EPA, 2011; Finley et al., 1994).

Only surface type was considered as a factor that influenced transfer: other characteristics like inoculum size, contact pressure, contact friction, and surface wetness were neglected in line with previous work (Jarvis et al., 2010; Julian and Pickering, 2015). When literature values were not found for surfaces observed in the video, transfer efficiency were assumed from existing literature values for similar surfaces. For example, transfer between hands and rice seeds was assumed to be similar to transfer between hands and loose granite (Table 1).

### 2.5.4. Surface areas

Distributions for surface areas in contact with the hands are based on the expected grip type and corresponding fractional surface area as described by AuYeung et al. (2008) (Table 2) (AuYeung et al., 2008; Beamer et al., 2015; U.S. EPA, 2011). Contact surfaces of the area of hand-to-mouth contact events are assumed to be partial finger immersions of between 2 and 3 fingers (Table 2). Surface areas of hands are assumed to be 910 cm<sup>2</sup>, the center point of the recommended range for women (760–1060 cm<sup>2</sup>) as the vast majority (96%) of farmers studied were women) (AuYeung et al., 2008; Beamer et al., 2015; U.S. EPA, 2011).

### 2.6. Dose assessment

Estimates of the *E. coli* ingested by the farmers based on the stochastic-mechanistic simulation are compared to models informed by assumptions of uniform exposure frequencies (Mattoli et al., 2015;

**Table 1**

Distributions of transfer efficiency used to model transfer of either *E. coli* or bulk material between objects (surfaces or bulk materials) and hands for each contact event. For bulk material transfer, *E. coli* transfer was modeled based on both the mass of bulk material transferred and the concentration of *E. coli* in the material. Parameters specified are mean and standard deviation for normal (N) distributions or minimum and maximum for uniform (U) distributions.

Category	Surface	Reference organism	Parameters	Reference
Surfaces (%)				
Bicycle	Stainless steel	<i>E. coli</i>	N(54,23)	Lopez et al. (2013)
Footwear	Rubber	<i>A. baumannii</i>	N(35,19)	Greene et al. (2015)
Cloth	Cotton	<i>E. coli</i>	N(13,12)	Lopez et al. (2013)
Door/wall	Laminate	<i>E. coli</i>	N(27,30)	Lopez et al. (2013)
Grass	Paper currency	<i>E. coli</i>	N(0.1, 0.3)	Lopez et al. 2013
Handheld tools	Laminate	<i>E. coli</i>	N(27,30)	Lopez et al. (2013)
Mask	Cotton	<i>E. coli</i>	N(13,12)	Lopez et al. (2013)
Phone	Phone	<i>S. rubidea</i>	N(38,10)	Rusin et al. (2002) (mean), Greene et al. (2015) (standard deviation)
Paper currency	Paper currency	<i>E. coli</i>	N(0.1, 0.3)	Lopez et al. (2013)
Bucket (plastic)	Polypropylene	<i>A. baumannii</i>	N(21,13)	Greene et al. (2015)
Polysacks bag	Polyester	<i>E. coli</i>	N(0.7,0.8)	Lopez et al. (2013)
Polyethene bag	Polypropylene	<i>A. baumannii</i>	N(21,13)	Greene et al. (2015)
Rice seeds	Granite	<i>E. coli</i>	N(37,39)	Lopez et al. (2013)
Toilet paper	Paper currency	<i>E. coli</i>	N(0.1, 0.3)	Lopez et al. (2013)
Toilet pit	Laminate	<i>E. coli</i>	N(27,30)	Lopez et al. (2013)
Water (ml/cm <sup>2</sup> )				
Water/surface	Water	–	U[0.00214–0.00499]	U.S. EPA (2011)
Water/drinking	Water	–	U[0.00214–0.00499]	U.S. EPA (2011)
Bulk materials (mg/cm <sup>2</sup> )				
Excreta	Soil	–	U[0.16–0.28]	U.S. EPA (2011)
Mud	Mud	–	N(0.49,0.54)	Finley et al. (1994)
Ash	Soil	–	U[0.16–0.28]	U.S. EPA (2011)
Body (%)				
Hands	Fingerpad	<i>A. baumannii</i>	N(33,12)	Greene et al. (2015)
Face	Fingerpad	<i>A. baumannii</i>	N(33,12)	Greene et al. (2015)
Mouth	Lip	<i>S. rubidea</i>	N(34,25)	Rusin et al. (2002) (mean), Julian et al. (2010) (standard deviation)



**Table 2**

Modeled *E. coli* contamination and surface area of contact events for the surface, bulk material, and body categories contacted during the videography. *E. coli* contamination probability distribution functions were based on microbial sample collection. Surface area probability distribution functions were based on expected grip type and corresponding fractional surface area of contact, as described by AuYeung et al. (2008). <sup>1</sup>Sample size (n) used to estimate distributions. <sup>2</sup>Parameters specified are log<sub>10</sub>-transformed mean and standard deviation for normal distributions (N (log<sub>10</sub>)) or minimum and maximum for uniform distributions. Resample refers to random selection with replacement of one of the observed values which are specified in the array. <sup>3</sup>Grip types are defined by AuYeung et al. (2008) with associated uniform distributions specifying minimum and maximum.

Category	n <sup>1</sup>	<i>E. coli</i> contamination <sup>2</sup>		Surface area <sup>3</sup>	
		Distribution	Parameters	Grip type	Parameters
Surfaces (CFU/100cm <sup>2</sup> )					
Bicycle	12	Resample	[0,0,0,0,0,0,360,5480]	Closed hand grip	[0.1, 0.17]
Cloth	9	N(log <sub>10</sub> )	(1.68, 0.75)	Front partial fingers	[0.04,0.06]
Door/wall	10	N(log <sub>10</sub> )	(2.48, 1.03)	Partial front palm w/ fingers	[0.07, 0.14]
Footwear	10	N(log <sub>10</sub> )	(1.94, 1.93)	Front partial fingers	[0.04,0.06]
Grass and rice seeds	10	N(log <sub>10</sub> )	(0.55, 1.50)	Partial front palm w/ fingers	[0.07, 0.14]
Handheld tools	10	N(log <sub>10</sub> )	(3.22, 1.21)	Closed hand grip	[0.1, 0.17]
Mask	11	Resample	[0,0,0,0,0,0,0, 200,240]	Front partial fingers	[0.04,0.06]
Phone	10	Resample	[0,0,0,0,0,0,0,720,1280]	Closed hand grip	[0.1, 0.17]
Paper currency	10	Point	0	Front partial fingers	[0.04,0.06]
Bucket (plastic)	10	N(log <sub>10</sub> )	(1.03, 1.21)	Closed hand grip	[0.1, 0.17]
Polysacks bag	23	N(log <sub>10</sub> )	(2.03, 1.70)	Front partial fingers	[0.04,0.06]
Polythene bag	10	Resample	[0,0,0,0,0,0,0,40,840,4000]	Front partial fingers	[0.04,0.06]
Toilet paper	10	Point	0	Front partial fingers	[0.04,0.06]
Toilet pit	10	N(log <sub>10</sub> )	(3.76, 1.04)	Open hand grip	[0.1,0.21]
	155				
Bulk materials ( <sup>1</sup> CFU/100 ml or <sup>2</sup> CFU/g-dry)					
Surface water <sup>f</sup>	24	N(log <sub>10</sub> )	(3.91, 0.84)	Partial finger immersion	[0.26, 0.30]
Domestic water <sup>f</sup>	32	N(log <sub>10</sub> )	(0.12, 0.79)	Partial finger immersion	[0.26, 0.30]
Excreta <sup>f</sup>	20	N(log <sub>10</sub> )	(4.00, 2.14)	Full front palm with fingers	[0.13, 0.25]
Mud <sup>f</sup>	20	N(log <sub>10</sub> )	(2.02, 1.40)	Full front fingers	[0.04,0.1]
Ash <sup>f</sup>	10	Resample	[0,0,0,0,0,8,9336, 657]	Full front palm with fingers	[0.13, 0.25]
	106				
Body (CFU/100cm <sup>2</sup> )					
Hands			From model	Closed hand grip	[0.1, 0.17]
Face		Point	0	Front partial fingers	[0.04,0.06]
Mouth			Not relevant	Partial finger immersion (2–3 fingers)	[0.10, 0.18]

Özkaynak et al., 2010). The arithmetic approach estimates dose assuming:

$$\text{Dose} = \sum_{k=1}^n (S * T * C_H) \quad (4)$$

where  $C_H$  is the concentration of bacteria on the hands (CFU/cm<sup>2</sup>) for the  $k$ th hand-to-mouth contact,  $T$  is the transfer efficiency of bacteria on contact (unitless),  $S$  is the fractional surface area of the hand (unitless), and  $n$  is the number of hand-to-mouth contact events per unit time (#/hr).

Four separate sets of assumptions about *E. coli* contamination on hands and frequency of hand-to-mouth contacts are compared to the stochastic-mechanistic simulation (Table 3). The goal of the comparison was to determine the impact of using the stochastic-mechanistic simulation presented here relative to the generalizable approach used elsewhere, and to determine the impact of assumptions about model parameters. For the comparison, other values (surface area and transfer) are assumed to be the same across all models (Table 3). Specifically, *E. coli* concentrations on the hands are described by either simulated

(Models 1 and 2) or measured (Models 3 and 4) *E. coli* concentrations. The simulated *E. coli* concentrations were determined by assuming a lognormal distribution describable by the *E. coli* concentrations across all farmers' hands at the end of the simulations (see Excreta collection and Land application). The measured *E. coli* concentrations were determined by assuming a lognormal distribution describable by the *E. coli* concentrations measured on the hands of the farmers (see Hands).

Probability distributions for frequency of hand-to-mouth contacts were based on either previously published estimates for adult hand-to-mouth contacts, or fit to data obtained from videography (Table 3). Published data were obtained from Jones (2011), who fit a Weibull distribution with shape of 0.76 and scale of 7.07 to observed adult hand-to-mouth contact data amongst United States office workers (Jones, 2011; Nicas and Best, 2008). Videographic data on hand-to-mouth contact frequency for each farmer were fit to a Weibull distribution (shape = 0.93, scale = 0.98) using the *fitdistcens* R package (R Core Team, 2016) (Table 3), with the data left censored at the limit of detection (1 contact per length of video) for farmers with no observed contacts.

**Table 3**

Alternative models used to estimate ingested *E. coli* dose based on the traditional arithmetic approach using *E. coli* hand contamination data estimated from our stochastic-mechanistic simulation (Models 1 and 2, see Results) or measured *E. coli* hand contamination (Models 3 and 4) and published (Models 1 and 3) or collected (Models 2 and 4) hand-to-mouth contact frequencies as compared to the stochastic-mechanistic simulation (Model 5). Parameters specified are log<sub>10</sub> mean and log<sub>10</sub> standard deviation for normal (N) and lognormal (LNORM) distributions; shape and scale for Weibull distributions; and minimum and maximum for uniform distributions. <sup>1</sup>Refers to Jones et al. (2011) reference.

Model	Surface area (S, cm <sup>2</sup> )	Transfer (T, %)	<i>E. coli</i> on hand (C <sub>H</sub> , CFU/cm <sup>2</sup> )		Freq. hand-mouth contacts (f <sub>H-M</sub> , #/hr)
			Collection	Application	
1	U[0.10, 0.18]	N(0.34,0.25)	Simulated: LNORM(1.4, 1.3)	Simulated: LNORM(0.8,1.5)	Published:Weibull (0.76, 7.07) <sup>1</sup>
2					Videography: Weibull (0.93, 0.98)
3			Measured: LNORM(−1.4,1.3)	Measured: LNORM(−0.4,1.2)	Published:Weibull (0.76, 7.07) <sup>1</sup>
4					Videography: Weibull (0.93, 0.98)
5			Stochastic-mechanistic model		

Dose assessment models are compared using cumulative probability distribution functions. The cumulative distribution function for the stochastic-mechanistic simulation was derived from aggregating final dose estimates for 100 simulations of each of the 13 (collection) or 15 (application) farmers (1300 and 1500 total simulations). The number of simulations was sufficient for convergence of estimates of *E. coli* contamination on hands (Supporting information, Fig. S7). The cumulative distribution functions for the arithmetic models are derived from the equivalent number of simulations: 1300 or 1500 for collection and application, respectively.

## 2.7. Sensitivity analysis

The simulation sensitivity analysis was conducted using a modified method of (Xue et al., 2006). In brief, parameter values (object contamination, transfer efficiency, and surface area) for each of the objects were set to the median (p50) point value of the probability distribution function, and the median *E. coli* concentrations on the hands of all farmers (exposure, in units of  $\log_{10}$  CFU/cm<sup>2</sup>) and the summed total *E. coli* ingested for all farmers (dose, in units of CFU) were calculated (Supporting information Table S2). Each parameter was then individually adjusted to the 10th (p10) or 90th (p90) percentile values based on the probability distribution function, and the corresponding exposure and dose were calculated. The percentage change in exposure and dose were then calculated using the ratios of p90:p50 and p50:p10, and the parameters were rank ordered by the magnitude of the percentage change.

Because the simulation used the MLATS data directly as opposed to drawing the data from a probability distribution function, a different approach was used to estimate impact of activity data on the simulation outcomes. Specifically, we assumed the observed activity frequency for each object category was equivalent to the median (p50) point value (obs., Supporting information Table S2). We defined the 10th (p10) and 90th (p90) percentile point values based on ordering the observed frequencies for the individual farmers. The relative impact of the activity frequency was determined by estimating the percentage change in the outcomes (exposure and dose) using the ratios p90:observed frequency and observed frequency:p10 (Supporting information Table S2).

## 3. Results

### 3.1. Excreta treatment

Of the 25 farmers enrolled in the study, 1 (4%) reported storing human excreta for <2 months, 9 (36%) reported <3 months, 12 (48%) reported <4 months, and 3 (12%) did not respond. No one reported storing human excreta for >6 months, the recommended minimum storage time (Mackie Jensen et al., 2008; Phuc et al., 2006; Winblad, 2004).

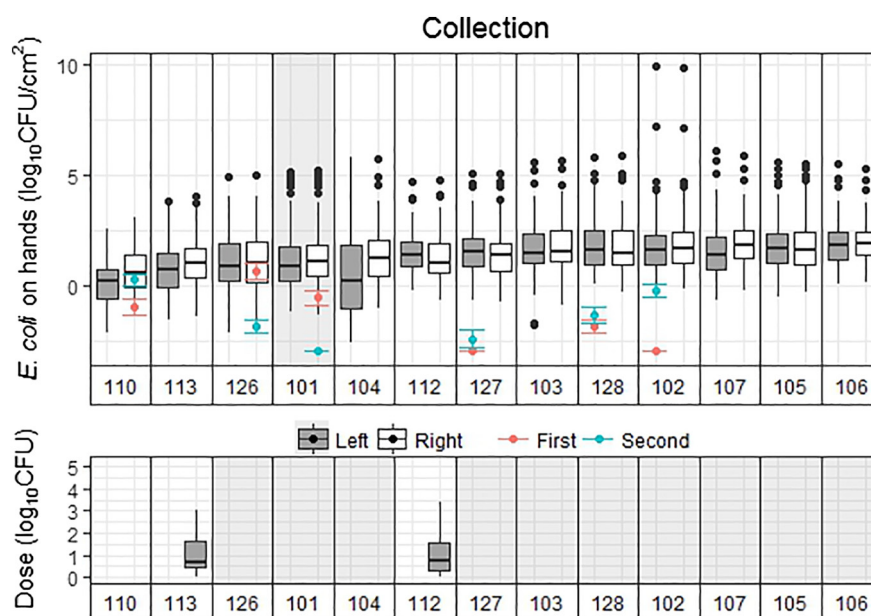
### 3.2. Parameter estimation

#### 3.2.1. Microbial contamination

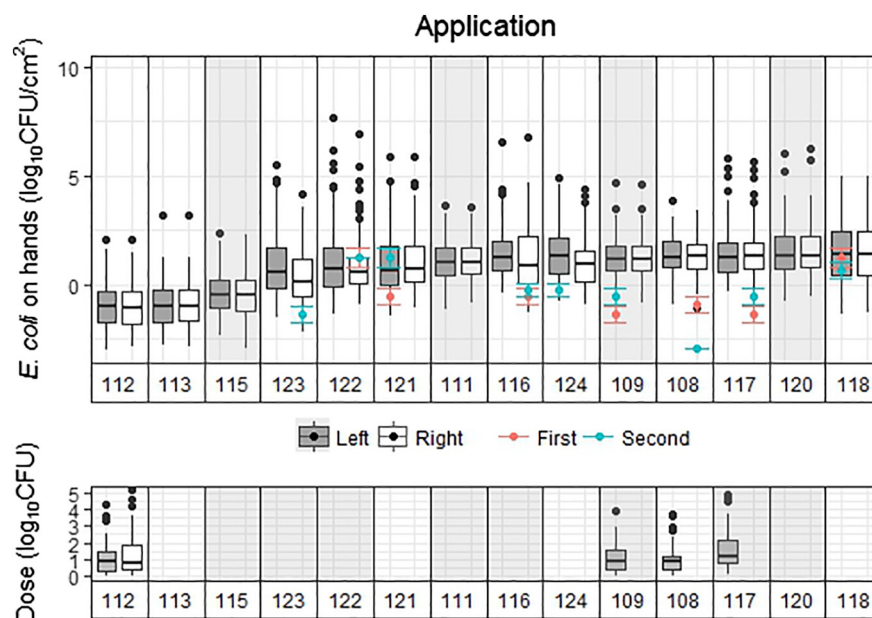
**3.2.1.1. Hands.** For all 30 hand samples collected (15 farmers, samples collected before and after videography), the average [standard deviation] of *E. coli* concentrations measured was 2.3 [1.2]  $\log_{10}$  MPN *E. coli* per hand. Four (13%) samples were below the limit of detection (<3 MPN *E. coli* per hand) and two (7%) samples were above the limit of detection (>10<sup>4</sup> MPN *E. coli* per hand) (Figs. 1 and 2).

Left hand contamination was not significantly different than right hand contamination (Wilcoxon rank sum test,  $p = 0.83$ ). Farmers collecting excreta had lower hand contamination than farmers applying excreta to fields (mean [standard deviation] 1.4 [1.1]  $\log_{10}$  MPN *E. coli* per hand as compared to 2.6 [1.1], Wilcoxon rank sum test,  $p = 0.01$ ).

**3.2.1.2. Surfaces.** Of the 155 surface samples collected, 81 (52%) were below the lower limit of detection (5 CFU/100 cm<sup>2</sup>). For the 14 surface categories, two (paper currency and toilet paper) had no detectable *E. coli* on any of the samples tested, and four (bicycle, mask, phone, and polyethylene bags) had 40% or fewer of the samples with detectable *E. coli* (Table 2). Contamination was modeled using sampling with replacement (Table 2). Microbial samples for the other eight surface categories (cloth, door/wall, footwear, grass and rice seeds, handheld tools, bucket (plastic), polysacks bag, and toilet pit) were fit to lognormal, base 10, distributions with means [standard deviations] ranging from 0.54 [1.54] (grass and rice seeds) to 3.76 [1.03] (toilet pit) (Table 2).



**Fig. 1.** Distributions from 100 simulations for final (top) *E. coli* hand contamination and (bottom) *E. coli* dose for farmers ( $n = 13$ ) collecting human excreta from latrines. Boxplots highlight median and interquartile ranges with whiskers extending up to 1.5 times the interquartile range beyond the median. Outliers are >1.5 times the interquartile range beyond the median. *E. coli* contamination on hands was measured for a subset ( $n = 6$ ) of farmers for either the left or right hand before (red) and after (blue) videography. Error bars represent standard deviations. The use of personal protective equipment by the farmers is noted by shading the background indicating (top) gloves or (bottom) masks. The number at the bottom of each figure indicate farmers' ID. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)



**Fig. 2.** Simulated distributions from 100 simulations for final (top) *E. coli* hand contamination and (bottom) *E. coli* dose for farmers ( $n = 14$ ) applying human excreta to land. Boxplots highlight median and interquartile ranges with whiskers extending up to 1.5 times the interquartile range beyond the median. Outliers are >1.5 times the interquartile range beyond the median. *E. coli* contamination on hands was measured for a subset ( $n = 9$ ) of farmers for either the left or right hand before (red) and after (blue) videography. Error bars represent standard deviations. The use of personal protective equipment by the farmers is noted by shading the background (top) indicating gloves and (bottom) indicating masks. The number at the bottom of each figure indicate farmers' ID. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

**3.2.1.3. Bulk materials.** Of the 106 bulk material samples collected, 28 (26%) were below the lower limit of detection. *E. coli* contamination of most materials (surface and domestic water, excreta, and mud) were fit to lognormal, base 10, distributions with means [standard deviations] ranging from 0.12 [0.79] CFU/100 ml (drinking water) to 4.00 [2.14] CFU/g (excreta) (Table 2). Because only 4/10 (40%) of ash samples contained detectable *E. coli*, ash contamination was modeled using sampling with replacement (Table 2).

### 3.2.2. Activity data

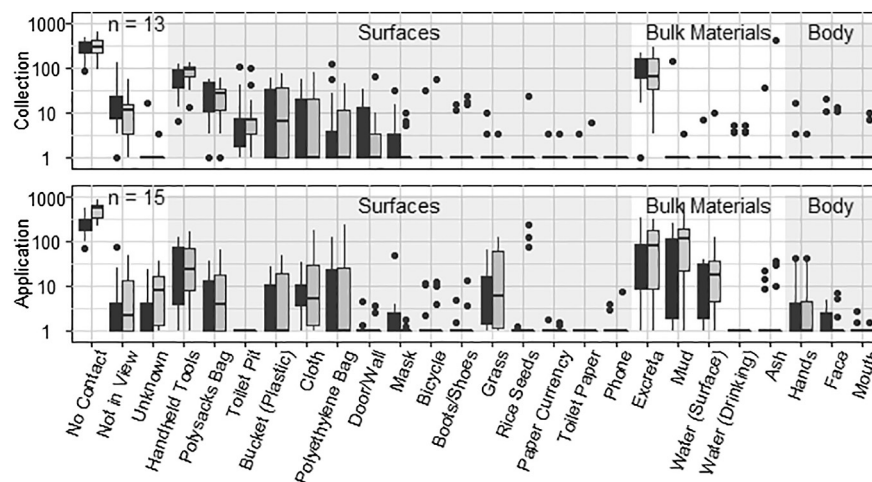
In total, 18.2 h of video were collected and translated from 25 farmers. Individual video lengths ranged from 0.17 to 1.62 h. Of the 18.2 h, 0.5 h was translated by both coders for comparison. The dual comparison showed approximately 15% deviation in frequency of the coding (see Supplemental information Table S1, Fig. S1). This is above the minimum recommended inter-observer agreement for video translation as described by Ferguson et al. (2006), but as shown by Sensitivity

Analysis (see Sensitivity Analysis) likely has no meaningful impact on outcomes (Ferguson et al., 2006).

The mean [standard deviation] of a farmer's left and right hand-object contacts were 360 [136] and 401 [284] times per hour, respectively, during collection of human excreta from dry toilets (Table 1). During land application of excreta, the left and right hands contacted an object 342 [198] and 848 [340] times per hour, respectively. The dramatically higher contact frequency of the right hand during land application is attributable to observed rapid, repeated, motions (i.e., spreading excreta, seeding).

The most common categories contacted during both excreta collection and land application were handheld tools (i.e., shovel, rakes), polysacks bag, and human excreta (Fig. 3). The toilet pit was frequently contacted only during excreta collection, while mud and surface waters were frequently contacted only during land application (Fig. 3).

Contacts with body parts (hand-to-hand, hand-to-face, and hand-to-mouth) were infrequent (Fig. 3, Table 4). Notably, only 2/14 (14%)



**Fig. 3.** Frequency of contacts (number of contacts per hour) observed for (a) excreta collection and (b) land application. Boxplots highlight median and interquartile ranges with whiskers extending up to 1.5 times the interquartile range beyond the median. Outliers are >1.5 times the interquartile range beyond the median.

**Table 4**

Frequency (number per hour) for contacts with anything (All Contacts), Hands, Face, and Mouth for the left and right hands of farmers during excreta collection and land application.

Activity	Hand	All Contacts		Hands		Face		Mouth	
		Mean (sd)	Median [range]	Mean (sd)	Median [range]	Mean (sd)	Median [range]	Mean (sd)	Median [range]
Collection	Left	360 (136)	342 [105, 579]	1.4 (4.4)	0 [0,15]	2.2 (5.8)	0 [0,19.3]	0 (0)	0 [0,0]
	Right	401 (284)	327 [146, 1256]	0.4 (0.9)	0 [0, 2.4]	1.6 (4.2)	0 [0,12.5]	1.1 (2.9)	0 [0,9.2]
Application	Left	342 (198)	262 [88,804]	7.3 (13.9)	0 [0, 40.5]	0.9 [1.4]	0 [0, 3.8]	0.3 (0.6)	0 [0,1.7]
	Right	848 (340)	880 [34, 1344]	7.5 (13.6)	0 [0,42.0]	0.8 (1.8)	0 [0, 6.0]	0 (0.1)	0 [0, 0.5]

and 3/15 (20%) of farmers contacted their mouths during excreta collection and land application, respectively. The observed frequency was much lower than the frequency estimated for office workers in the U.S (Jones, 2011; Nicas and Best, 2008). Probability distribution functions of the frequency of hand-to-mouth contacts observed for farmers in Vietnam (Weibull distribution, shape = 0.93, scale = 0.98) as compared to the probability distribution function for U.S. office workers (Weibull distribution, shape = 0.76, scale = 7.07) highlight the disparity (Fig. 4). This is notable because hand-to-mouth contacts are primarily route for ingested dose, and therefore primarily responsible for risk from pathogen exposures.

Personal protective equipment, specifically use of masks, was unexpectedly high. During excreta collection, 1/13 (8%) of farmers used a glove and 10/13 (77%) used a mask (Fig. 1, shading). During land application, 4/14 (29%) of farmers used gloves and 8/14 (57%) used masks (Fig. 2, shading).

### 3.3. Exposure simulation

*E. coli* concentrations were simulated for both the left and right hands of 25 farmers, 11 who collected excreta, 12 who applied excreta to land, and 2 who both collected and applied excreta. *E. coli* concentrations ranged from  $<10^{-4}$  to  $10^{10}$  CFU/cm<sup>2</sup> over 100 simulations for all 25 farmers (Supporting information, Fig. S2). For example, the *E. coli* contamination on the left and right hands of Farmer ID 112 during land application varied between  $<10^{-2.5}$  to  $10^5$  CFU/cm<sup>2</sup> over 100 simulations (Fig. 5). *E. coli* contamination on hands is dynamic, with large variation over time, as well as between different farmers.

#### 3.3.1. Excreta collection

Overall, the simulation (100 simulations per farmer) predicted final *E. coli* concentrations across all farmers for both hands of mean [standard deviation] of 1.4 [1.3] log<sub>10</sub> CFU/cm<sup>2</sup> during excreta collection as shown in Fig. 1. Concentrations for left and right hands ranged across farmers from median [interquartile range, IQR] of 0.2 [−0.7,0.7] and 0.6 [−0.1,1.4] log<sub>10</sub> CFU respectively, for Farmer ID 110 to 1.9 [1.2,2.4] and 1.8 [1.4,2.4] log<sub>10</sub> CFU, respectively, for Farmer ID 106 (Fig. 1). The simulated median *E. coli* concentrations on hands were statistically significantly higher than measured *E. coli* concentrations by an average

[standard deviation] of 2.1 [1.5] log<sub>10</sub> CFU (n = 15, p = 0.0003, paired Wilcoxon rank sum test).

Only two farmers (Farmers 112 and 113) contacted their mouths (2 and 3 times, respectively), resulting in estimated *E. coli* ingestion of median [IQR] of 2.0 [0.5,10.0] and 0.7 [0.1, 4.2] CFU (Fig. 1).

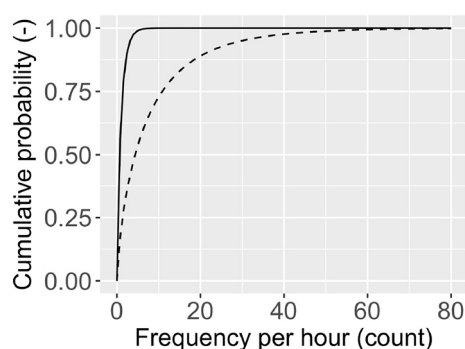
#### 3.3.2. Land application

The simulation of *E. coli* contamination on hands after land application predicted concentrations across all farmers, for both hands, of mean (standard deviation) 0.8 (1.5) log<sub>10</sub> CFU/cm<sup>2</sup> as shown in Fig. 2. Concentrations for left and right hands on farmers ranged from median [IQR] of −1.1 [−1.9, −0.4] and −1.2 [−2.0, −0.4] log<sub>10</sub> CFU respectively (Farmer 112) to 1.4 [0.4,2.4] and 1.3 [0.4, 2.4] log<sub>10</sub> CFU, respectively (Farmer 118). The estimated median *E. coli* concentrations on hands were statistically significantly higher than measured *E. coli* concentrations by an average [standard deviation] of 1.3 (1.5) log<sub>10</sub> CFU (p = 0.03, paired samples *t*-test). Simulated *E. coli* concentrations following land application were statistically significantly less than simulated concentrations following excreta collections (p < 0.001, *t*-test), which was the reverse of what was observed for measured *E. coli* concentrations (see Hands).

#### 3.4. Dose assessment

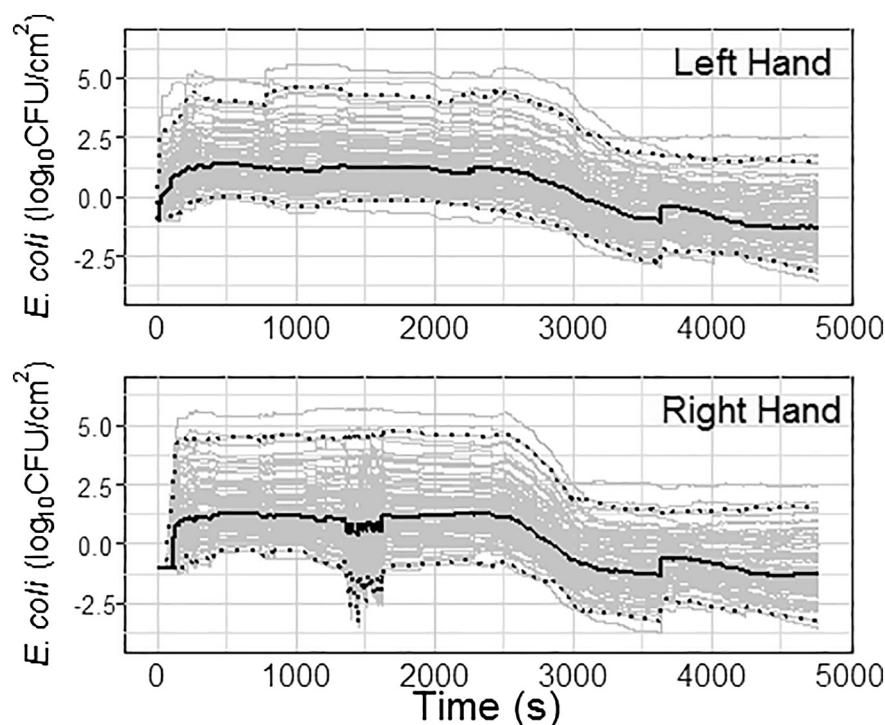
Within the stochastic-mechanistic simulation, the majority of farmers (20/25, or 80%) did not have an observed hand-to-mouth contact event, and so did not ingest *E. coli* during observation (Figs. 1 and 2). Of the remaining five farmers, two (Farmers 112 and 113) contacted their mouths during excreta collection, resulting in estimated *E. coli* ingestion of mean [95% Confidence Interval] 3.1 [1.9, 4.8] and 0.9 [0.5, 1.6] CFU (Fig. 1). Normalized to length of video, this corresponds to 10.4 [6.5, 16.4] and 3.2 [1.9, 5.5] CFU/hr. Four farmers (Farmers 108, 109, 112 and 117) contacted their mouths during land application, resulting in estimated *E. coli* ingestion of mean [95% Confidence Interval] of 1.2 [0.7,2], 4.2 [2.7,6.5], 3.0 [1.6,6.1], and 0.6 [0.3,1.3] CFU, respectively (Fig. 2). Normalized to video length, this corresponds to 0.7 [0.4,1.3], 2.9 [1.8,4.4], 2.2 [1.2,4.4], and 0.7 [0.3,1.5] CFU/h. Notably, during Land Application, two of the farmers with hand-to-mouth contacts (109 and 117) wore masks for some, but not all, of the recording.

Dose estimates using distinct assumptions about parameter values highlight the impact of exposure and concentration data sources on estimated exposures (Fig. 6). The arithmetic models (Models 1–4) generally assume more frequent low dose exposures than the stochastic-mechanistic simulation (Model 5). Dose estimates are higher when simulated *E. coli* hand contamination (Models 1,2) is used as compared to measured *E. coli* hand contamination (Models 3,4), reflecting the overestimation of the stochastic-mechanistic simulation (see Exposure simulation). Similarly, dose estimates are higher when published hand-to-mouth frequencies are used (Models 1,3, see Table 3 and Fig. 4 for the two probability distribution functions (published and observed) as compared to observed hand-to-mouth frequencies (Models 2,4). Notably, for doses above 0.1 CFU *E. coli*/hr, the stochastic-mechanistic simulation (Model 5) aligned with the arithmetic model using observed hand-to-mouth frequency data with measured *E. coli* contamination data (Model 4).



**Fig. 4.** Cumulative probability of hand-to-mouth frequency per hour from the observed in this study (solid line) and the published by Jones (2011) (dashed line).





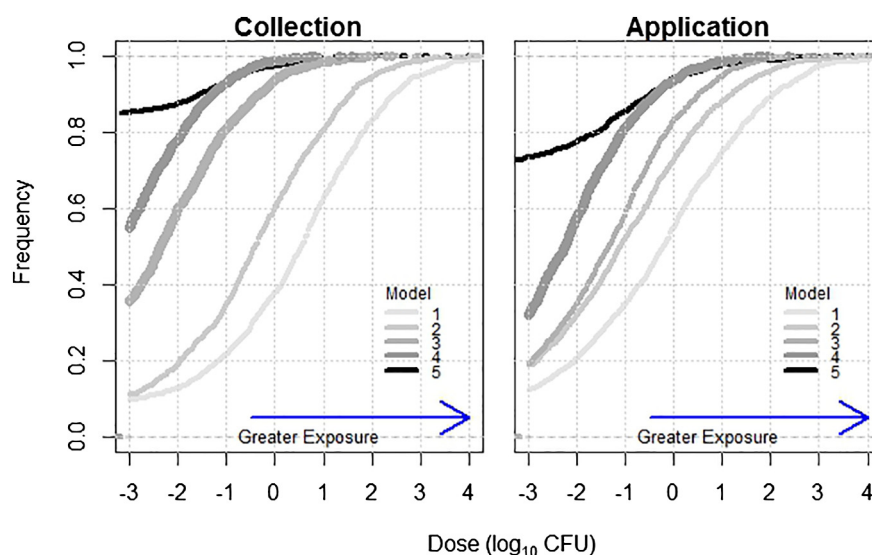
**Fig. 5.** Median (solid, black) and 95% range (dashed, black) *E. coli* concentrations on (top) left and (bottom) right hands of Farmer ID 112 during land application of excreta to agricultural fields from 100 simulations (solid, gray).

### 3.5. Sensitivity analysis

Sensitivity analysis highlighted the importance of *E. coli* contamination in the environment (specifically, *E. coli* contamination of excreta, handheld tools, and the toilet pit) on both *E. coli* contamination of the hands (exposure) and *E. coli* ingested (dose) (Table 5, Figs. S3–S6). Activity data were also influential for *E. coli* contamination of hands (specifically the frequency of contacts with excreta, the toilet pit, or surface water) and *E. coli* ingested (the frequency of hand-to-mouth contacts). Neither surface area nor transfer efficiency influenced model outputs (Table 5, Figs. S3–S6).

### 4. Discussion

The study highlights substantial inter-individual variation in *E. coli* on hands and ingested *E. coli* for twenty-five farmers using human excreta for agriculture in Vietnam. The large variation is attributed to microbial contamination in the environment and hand-to-mouth contact frequency. Specifically, *E. coli* contamination of excreta and other frequently contacted objects (i.e., handheld tools, toilet pits) strongly influenced hand contamination. *E. coli* contamination of excreta and hand-to-mouth contact frequency influenced ingested dose. Notably, hand-to-mouth contact frequency was substantially less than previously



**Fig. 6.** Empirical cumulative distribution functions (ECDF) for the estimated ingested *E. coli* dose ( $\log_{10}$  CFU) over 1 h as modeled using arithmetic models based on (Model 1) simulated *E. coli* hand contamination and literature values for hand-to-mouth frequency, (Model 2) simulated *E. coli* hand contamination and observed hand-to-mouth frequency, (Model 3) measured *E. coli* hand contamination and literature values for hand-to-mouth frequency, and (Model 4) measured *E. coli* hand contamination and observed hand-to-mouth frequency data, as compared to (Model 5) stochastic-mechanistic exposure and dose simulation. Line thickness is an artifact of the step-function used to derive the ECDF. See Table 3 for model details.

observed in other settings. Our findings align with an epidemiological study showing increased risks for farmers composting excreta for <3 months (paralleling high pathogen concentrations), and not using PPE or never or rarely washing hands with soap (paralleling impact of frequent hand-to-mouth exposures) (Pham-Duc et al., 2014).

Control of human excreta through adequate treatment (i.e., addition of lime or ash, sufficient storage time) to reduce pathogen concentrations is likely the most effective intervention for excreta land application, as the sensitivity analysis of the impact of *E. coli* concentrations of human excreta on dose demonstrated. In our simulation, shifting estimated *E. coli* contamination of human excreta over the range of *E. coli* concentration values observed from microbial sampling dramatically influenced ingested *E. coli* estimates. Other potential control options such as PPE, though promising, likely have limited efficacy. PPE's primary role is to reduce exposure and dose by impacting hand-to-mouth contacts. However, we observed that PPE did not always prevent against hand-to-mouth contact events due to imperfect compliance. Two farmers (Farmer IDs 109 and 117) used masks but still ingested *E. coli*. Furthermore, containment of excreta is also likely an effective intervention for excreta collection. Our simulation showed the highest sensitivity of toilet pit *E. coli* concentration on the dose during collection.

Surprisingly, hand contamination was higher during land application than excreta collection. This is likely due to more frequent contacts with *E. coli* from media other than human excreta, such as handheld tools, polysacks bags, mud, and surface or irrigation water. This assertion is supported in our finding that *E. coli* contamination on hands was influenced by *E. coli* contamination of many more media during application than during collection. It is possible that some of these other media may contain *E. coli* from non-human sources, such as from animals and/or growth in the environment (Ishii et al., 2010, 2006). Studies employing pathogen detection and/or source tracking assays may be

better positioned to estimate the relative risks of excreta collection as compared to land application.

Exposure and risk assessments should incorporate high quality human-environment interaction data. The observed hand-to-mouth contact frequency obtained from videography for farmers was substantially lower than the hand-to-mouth contact frequency reported in the literature for adults (Jones, 2011; Nicas and Best, 2008). The study populations (Vietnamese Farmers and United States Office Workers) and target actions (excreta use and office work) are vastly different, and so it is unsurprising that exposure factors are also vastly different. As the farmers likely recognize a risk of excreta use to some degree, it is reasonable that excreta users may be more considerate of risks from hand-to-mouth contacts, resulting in the lower frequency observed. Increasingly, studies are highlighting differences in exposure factors amongst different study populations, especially when comparing high income populations to low or middle income country populations (Kwong et al., 2016; Phillips and Moya, 2013). Here, we provide evidence that context-specific, evidence-based, data for exposure assessments is needed. Risk assessment studies reliant on exposure factors data obtained from different contexts and/or expert opinion likely underestimate the uncertainty associated with assumed exposure factors.

The stochastic-mechanistic simulation of hand contamination provides insights into the potential dynamism of *E. coli* contamination on hands. Simulated *E. coli* contamination on hands frequently shifts during the observation period. The simulation dynamics mirror findings from experimental studies showing rapid temporal variability of *E. coli* and other bacteria on hands (Pickering et al., 2011; Ram et al., 2011). Additionally, the simulation suggests hand contamination is linked to microbial contamination in the environment: wide variation between simulations is largely attributable to variation in microbial contamination on objects. This finding also aligns with recent field work showing correlations between *E. coli* on hands and *E. coli* in household soils

**Table 5**

Sensitivity analysis rank and value for a subset of parameters used in the simulation of *E. coli* contamination on hands (Exposure) and *E. coli* ingested (Dose) for both collection of human excreta (Collection) and application on agricultural land (Application). The subset of parameters shown represent parameters that increased or decreased exposure or dose by >30% relative to the median (p50) simulation values (see Table S2).

Parameter	Exposure				Dose			
	Collection		Application		Collection		Application	
	Rank	Value	Rank	Value	Rank	Value	Rank	Value
Concentration								
Excreta								
<i>p50:p10</i>			3	0.98				
<i>p90:p50</i>	3	0.59	1	5.45	2	2389	1	3557
Handheld tools								
<i>p50:p10</i>			2	1.04				
<i>p90:p50</i>	1	2.46			3	16.1	2	65
Toilet pit								
<i>p90:p50</i>	4	0.5			1	5129		
Polysacks bag								
<i>p90:p50</i>			4	0.76				
Mud								
<i>p90:p50</i>			5	0.63				
Cloth								
<i>p90:p50</i>			6	0.59				
Water/surface								
<i>p90:p50</i>			7	0.52				
Bucket								
<i>p90:p50</i>			8	0.39				
Activity								
Mouth								
<i>p50:p10</i>					5	1.0	4	1.0
<i>p90:p50</i>					4	3.8	3	3.0
Excreta								
<i>p50:p10</i>	2	0.61						
Toilet pit								
<i>p90:p50</i>	5	0.34						
Water/surface								
<i>p90:p50</i>			8	0.4				

(Julian et al., 2013; Navab-Daneshmand et al., n.d.). This is additionally important given frequent reports of *E. coli* contamination on surfaces in households throughout low and middle income countries (Ercumen et al., 2017; Julian et al., 2013; Navab-Daneshmand et al., n.d.; Pickering et al., 2012; Sinclair and Gerba, 2011).

The simulation substantially overestimates hand contamination relative to measured *E. coli* data. *E. coli* contamination of surfaces and bulk materials was measured at different times than videography and hand samples, so it is possible that the environmental surfaces sampled were more contaminated than the surfaces the farmers contacted during videography. Another potential source of bias is the simulation assumption that *E. coli* contamination on hands is uniformly distributed. In reality, *E. coli* on hands is likely heterogeneous, and areas with more frequent surface contacts (i.e., tips of fingers, front of hands) may have relatively higher local *E. coli* contamination.

Another potential cause for overestimation includes inaccurate curve fitting of *E. coli* contamination data. Collected *E. coli* contamination data included high rates of non-detects. When data were fit to probability distribution functions, non-detects were assumed to be censored below the limit of detection. Maximum likelihood estimation of the probability distribution functions models non-detects as contaminated at levels below the limit of detection. It is possible that these surfaces were not contaminated at all. Assuming any contamination, even low levels, would lead to overestimation relative to assumptions of no contamination. Sampling and analytical methods may need to be modified to improve data at the tails of the probability distribution functions. Finally, the simulation assumes the proportion of *E. coli* transferred on contact is a function of the gradient in *E. coli* contamination of the two surface areas in contact. This assumption is not grounded in experimental literature, as transfer efficiency studies overwhelmingly study transfer from a contaminated to uncontaminated surface (Julian et al., 2010; Lopez et al., 2013; Rusin et al., 2002). More studies are needed to determine transfer dynamics between two contaminated surfaces.

Notable study limitations include the potential for introducing bias into behaviours of the farmers, and the lack of coincident sampling, both of which may have influenced the simulation outcomes. As the farmers were asked to enroll in a study observing their behaviours, it is highly likely that their behaviours were influenced during observation (e.g., reactivity). The high proportion of personal protective equipment and relatively low frequency of hand-to-mouth contacts may be at least partially attributable to reactivity. In the context of handwashing, for example, Ram et al. (2010) demonstrate study participant reactivity in the presence of an observer (Ram et al., 2010). Another source of bias, as previously discussed, is the collection of videography and hand sampling data at different times, which may have introduced bias into the simulation.

## 5. Conclusions

The primary finding from the study is that there is substantial inter-individual variation in *E. coli* hand contamination and ingested dose amongst farmers in Vietnam reliant on human excreta for agriculture. Additional findings include:

- Frequency of hand-to-mouth contacts amongst Vietnamese farmers substantially lower than the widely-used exposure factor previously reported for U.S. office workers.
- Variation in exposure and dose is driven by microbial contamination and frequency of hand-to-mouth contacts.
- Stochastic-mechanistic simulation is beneficial in that it highlights the dynamism of *E. coli* contamination on hands
- However, the simulation performs similar to simpler arithmetic models for estimating population-level exposures, when context-specific exposure factors are used.
- Exposure assessments should collect and integrate context-specific exposure factors to improve exposure and risk estimates.

- In Vietnam, intervention strategies should focus on reducing pathogen contamination of human excreta and handheld tools and/or prevent hand-to-mouth contacts.
- Personal protective equipment, though beneficial, is not completely protective due to imperfect use.

## Acknowledgements

This work contains portions of the Master of Science degree thesis for Hasitha S.K. Vithanage at the UNESCO-IHE Institute for Water Education (Delft, Netherlands), Min Li Chua at the Graduate School of Global Environmental Studies, Kyoto University (Kyoto, Japan), and of the the Master of Engineering degree thesis for Masataka Kuroda at the Graduate School of Engineering, Kyoto University. The work was supported by JSPS KAKENHI Grant [JP16H04436], and the Research Institute for Humanity and Nature Fund [14200107]. Hasitha S. K. Vithanage was supported by Eawag through the Eawag Partnership Program for Developing Countries 2015 Fellowship.

## Appendix A. Supporting Information

Supporting information to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2018.04.100>.

## References

- Amha, Y.M., Kumaraswamy, R., Ahmad, F., 2015. A probabilistic QMRA of Salmonella in direct agricultural reuse of treated municipal wastewater. *Water Sci. Technol.* 71, 1203–1211.
- AuYeung, W., Canales, R.A., Leckie, J.O., 2008. The fraction of total hand surface area involved in young children's outdoor hand-to-object contacts. *Environ. Res.* 108, 294–299.
- Beamer, P.L., Canales, R.A., Bradman, A., Leckie, J.O., 2009. Farmworker children's residential non-dietary exposure estimates from micro-level activity time series. *Environ. Int.* 35, 1202–1209.
- Beamer, P.L., Plotkin, K.R., Gerba, C.P., Sifuentes, L.Y., Koenig, D.W., Reynolds, K.A., 2015. Modeling of human viruses on hands and risk of infection in an office workplace using micro-activity data. *J. Occup. Environ. Hyg.* 12, 266–275.
- Blumenthal, U., Peasey, A., 2002. Critical Review of Epidemiological Evidence of the Health Effects of Wastewater and Excreta Use in Agriculture. World Health Organization.
- Cordell, D., Rosemarin, A., Schröder, J.J., Smit, A.L., 2011. Towards global phosphorus security: a systems framework for phosphorus recovery and reuse options. *Chemosphere* 84, 747–758.
- Do, T.T., Mølbak, K., Phung, D.C., Dalsgaard, A., 2007. Helminth infections among people using wastewater and human excreta in peri-urban agriculture and aquaculture in Hanoi, Vietnam. *Tropical Med. Int. Health* 12 (Suppl. 2), 82–90.
- Ercumen, A., Pickering, A.J., Kwong, L.H., Arnold, B.F., Parvez, S.M., Alam, M., Sen, D., Islam, S., Kullmann, C., Chase, C., Ahmed, R., Unicomb, L., Luby, S.P., Colford Jr., J.M., 2017. Animal feces contribute to domestic fecal contamination: evidence from *E. coli* measured in water, hands, food, flies, and soil in Bangladesh. *Environ. Sci. Technol.* 51, 8725–8734.
- Ferguson, A.C., Canales, R.A., Beamer, P., Auyeung, W., Key, M., Munninghoff, A., Lee, K.T.-W., Robertson, A., Leckie, J.O., 2006. Video methods in the quantification of children's exposures. *J. Expo. Sci. Environ. Epidemiol.* 16, 287–298.
- Ferguson, A., Canales, R., Vieira, V., Leckie, J., 2013. Methodology to capture children's non-dietary ingestion exposure activities during meal events. *Hum. Ecol. Risk Assess.* 19, 944–958.
- Finley, B., Proctor, D., Scott, P., Harrington, N., Paustenbach, D., Price, P., 1994. Recommended distributions for exposure factors frequently used in health risk assessment. *Risk Anal.* 14, 533–553.
- Fuhrmann, S., Winkler, M.S., Stalder, M., Niwagaba, C.B., Babu, M., Kabatereine, N.B., Halage, A.A., Utzinger, J., Cissé, G., Nauta, M., 2016. Disease burden due to gastrointestinal pathogens in a wastewater system in Kampala, Uganda. *Microbial. Risk Anal.* 4, 16–28.
- Fuhrmeister, E.R., Schwab, K.J., Julian, T.R., 2015. Estimates of nitrogen, phosphorus, biochemical oxygen demand, and fecal coliforms entering the environment due to inadequate sanitation treatment technologies in 108 low and middle income countries. *Environ. Sci. Technol.* 49, 11604–11611.
- Genthe, B., Rodda, N., 1999. Application of Health Risk Assessment Techniques to Microbial Monitoring Data. WRC Report No. 9611/99. Water Research Commission, Pretoria, South Africa.
- Giang, P.H., Harada, H., Fujii, S., Lien, N.P.H., Hai, H.T., Tanaka, S., Kunacheva, C., 2012. Waste and wastewater management and its impacts in a sub-urban community in Hanoi, Vietnam: a nitrogen and phosphorus flow analysis. *J. Jpn. Soc. Civil Eng., Ser. G (Environ. Res.)* 68 (III\_741–III\_749).
- Giang, P.H., Harada, H., Fujii, S., Lien, N.P.H., Hai, H.T., Anh, P.N., Tanaka, S., 2015. Transition of fertilizer application and agricultural pollution loads: a case study in the Nhue-Day River basin. *Water Sci. Technol.* 72, 1072–1081.

- Greene, C., Vadlamudi, G., Eisenberg, M., Foxman, B., Koopman, J., Xi, C., 2015. Fomite-fingerpad transfer efficiency (pick-up and deposit) of *Acinetobacter baumannii*—with and without a latex glove. *Am. J. Infect. Control* 43, 928–934.
- Gretsch, S.R., Ampofo, J.A., Baker, K.K., Clennon, J., Null, C.A., Peprah, D., Reese, H., Robb, K., Teunis, P., Wellington, N., Yakubu, H., Moe, C.L., 2016. Quantification of exposure to fecal contamination in open drains in four neighborhoods in Accra, Ghana. *J. Water Health* 14, 255–266.
- Haas, C.N., Rose, J.B., Gerba, C.P., 2014. *Quantitative Microbial Risk Assessment*. Hoboken, New Jersey, USA.
- Heinonen-Tanski, H., Helvi, H.-T., van Wijk-Sijbesma, C., 2005. Human excreta for plant production. *Bioresour. Technol.* 96, 403–411.
- Ishii, S., Ksoll, W.B., Hicks, R.E., Sadowsky, M.J., 2006. Presence and growth of naturalized *Escherichia coli* in temperate soils from Lake Superior watersheds. *Appl. Environ. Microbiol.* 72, 612–621.
- Ishii, S., Yan, T., Vu, H., Hansen, D.L., Hicks, R.E., Sadowsky, M.J., 2010. Factors controlling long-term survival and growth of naturalized *Escherichia coli* populations in temperate field soils. *Microbes Environ.* 25, 8–14.
- Jarvis, B., Wilrich, C., Wilrich, P.-T., 2010. Reconsideration of the derivation of Most Probable Numbers, their standard deviations, confidence bounds and rarity values. *J. Appl. Microbiol.* 109, 1660–1667.
- Jensen, P.K.M., Phuc, P.D., West, L.G.K., 2010. How do we sell the hygiene message? With dollars, dong or excreta? *Environ. Health* 9, 27.
- Jones, R.M., 2011. Critical review and uncertainty analysis of factors influencing influenza transmission. *Risk Anal.* 31, 1226–1242.
- Julian, T.R., Pickering, A.J., 2015. A pilot study on integrating videography and environmental microbial sampling to model fecal bacterial exposures in peri-urban Tanzania. *PLoS One* 10, e0136158.
- Julian, T.R., Canales, R.A., Leckie, J.O., Boehm, A.B., 2009. A model of exposure to rotavirus from nondietary ingestion iterated by simulated intermittent contacts. *Risk Anal.* 29, 617–632.
- Julian, T.R., Leckie, J.O., Boehm, A.B., 2010. Virus transfer between fingerpads and fomites. *J. Appl. Microbiol.* 109, 1868–1874.
- Julian, T.R., MacDonald, L.H., Guo, Y., Marks, S.J., Kosek, M., Yori, P.P., Pinedo, S.R., Schwab, K.J., 2013. Fecal indicator bacteria contamination of fomites and household demand for surface disinfection products: a case study from Peru. *Am. J. Trop. Med. Hyg.* 89, 869–872.
- Julian, T.R., Bustos, C., Kwong, L.H., Badilla, A.D., Lee, J., Bischel, H.N., Canales, R.A., n.d. Quantifying Human-Environment Interactions Using Videography in the Context of Infectious Disease Transmission. (Submitted).
- Knudsen, L.G., Phuc, P.D., Hiep, N.T., Samuelsen, H., Jensen, P.K., Dalsgaard, A., Raschid-Sally, L., Konradsen, F., 2008. The fear of awful smell: risk perceptions among farmers in Vietnam using wastewater and human excreta in agriculture. *Southeast Asian J. Trop. Med. Public Health* 39, 341–352.
- Kotloff, K.L., Nataro, J.P., Blackwelder, W.C., Nasrin, D., Farag, T.H., Panchalingam, S., Wu, Y., Sow, S.O., Sur, D., Breiman, R.F., Faruque, A.S., Zaidi, A.K., Saha, D., Alonso, P.L., Tamboura, B., Sanogo, D., Onwuchekwa, U., Manna, B., Ramamurthy, T., Kanungo, S., Ochieng, J.B., Omore, R., Oundo, J.O., Hossain, A., Das, S.K., Ahmed, S., Qureshi, S., Quadri, F., Adegbola, R.A., Antonio, M., Hossain, M.J., Akinsola, A., Mandomando, I., Nhamposha, T., Acácio, S., Biswas, K., O'Reilly, C.E., Mintz, E.D., Berkeley, L.Y., Muhsen, K., Sommerfelt, H., Robins-Browne, R.M., Levine, M.M., 2013. Burden and aetiology of diarrhoeal disease in infants and young children in developing countries (the Global Enteric Multicenter Study, GEMS): a prospective, case-control study. *Lancet* 382, 209–222.
- Kwong, L.H., Ercumen, A., Pickering, A.J., Unicomb, L., Davis, J., Luby, S.P., 2016. Hand- and object-mouthing of rural Bangladeshi children 3–18 months old. *Int. J. Environ. Res. Public Health* 13.
- Lam, S., Nguyen-Viet, H., Tuyet-Hanh, T.T., Nguyen-Mai, H., Harper, S., 2015. Evidence for public health risks of wastewater and excreta management practices in Southeast Asia: a scoping review. *Int. J. Environ. Res. Public Health* 12, 12863–12885.
- Langergraber, G., Muellegger, E., 2005. Ecological sanitation—a way to solve global sanitation problems? *Environ. Int.* 31, 433–444.
- Lopez, G.U., Gerba, C.P., Tamimi, A.H., Kitajima, M., Maxwell, S.L., Rose, J.B., 2013. Transfer efficiency of bacteria and viruses from porous and nonporous fomites to fingers under different relative humidity conditions. *Appl. Environ. Microbiol.* 79, 5728–5734.
- Mackie Jensen, P.K., Jensen, P.K.M., Phuc, P.D., Knudsen, L.G., Dalsgaard, A., Konradsen, F., 2008. Hygiene versus fertiliser: the use of human excreta in agriculture – a Vietnamese example. *Int. J. Hyg. Environ. Health* 211, 432–439.
- Mattioli, M.C.M., Davis, J., Boehm, A.B., 2015. Hand-to-mouth contacts result in greater ingestion of feces than dietary water consumption in Tanzania: a quantitative fecal exposure assessment model. *Environ. Sci. Technol.* 49, 1912–1920.
- Navab-Daneshmand, T., Friedrich, M.N.D., Gächter, M., Montealegre, M.C., Mlambo, L.S., Nhwitiwa, T., Mosler, H.-J., Julian, T.R., n.d. *E. coli* Contamination Across Multiple Environmental Reservoirs (Soils, Hands, Drinking Water, and Handwashing Water) in Urban Harare: Correlations and Risk Factors. (Submitted).
- Nicas, M., Best, D., 2008. A study quantifying the hand-to-face contact rate and its potential application to predicting respiratory tract infection. *J. Occup. Environ. Hyg.* 5, 347–352.
- Özkaynak, H., Xue, J., Zartarian, V.G., Glen, G., Smith, L., 2010. Modeled estimates of soil and dust ingestion rates for children. *Risk Anal.* 31, 592–608.
- Pham Duc, P., Duc, P.P., Nguyen-Viet, H., Hattendorf, J., Zinsstag, J., Cam, P.D., Odermatt, P., 2011. Risk factors for *Entamoeba histolytica* infection in an agricultural community in Hanam province, Vietnam. *Parasit. Vectors* 4, 102.
- Pham-Duc, P., Phuc, P.-D., Hung, N.-V., Jan, H., Cam, P.D., Christian, Z., Jakob, Z., Peter, O., 2014. Diarrhoeal diseases among adult population in an agricultural community Hanam province, Vietnam, with high wastewater and excreta re-use. *BMC Public Health* 14.
- Phillips, L.J., Moya, J., 2013. Exposure factors resources: contrasting EPA's Exposure Factors Handbook with international sources. *J. Expo. Sci. Environ. Epidemiol.* 24, 233–243.
- Phuc, P.D., Konradsen, F., Phuon, P.T., Cam, P.D., Dalsgaard, A., 2006. Practice of using human excreta as fertilizer and implications for health in Nghean Province, Vietnam. *Southeast Asian J. Trop. Med. Public Health* 37, 222–229.
- Pickering, A.J., Julian, T.R., Mamuya, S., Boehm, A.B., Davis, J., 2011. Bacterial hand contamination among Tanzanian mothers varies temporally and following household activities. *Tropical Med. Int. Health* 16, 233–239.
- Pickering, A.J., Julian, T.R., Marks, S.J., Mattioli, M.C., Boehm, A.B., Schwab, K.J., Davis, J., 2012. Fecal contamination and diarrheal pathogens on surfaces and in soils among Tanzanian households with and without improved sanitation. *Environ. Sci. Technol.* 46, 5736–5743.
- R Core Team, 2016. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL: <https://www.R-project.org/>.
- Ram, P.K., Sirajul Islam, M., Granger, S.P., Wright, R., Hitchcock, D., Jones, T., Nygren, B., Molyneaux, J.W., Halder, A.K., Hall, P., Luby, S.P., 2010. Is structured observation a valid technique to measure handwashing behavior? Use of acceleration sensors embedded in soap to assess reactivity to structured observation. *Am. J. Trop. Med. Hyg.* 83, 1070–1076.
- Ram, P.K., Jahid, I., Halder, A.K., Nygren, B., Islam, M.S., Granger, S.P., Molyneaux, J.W., Luby, S.P., 2011. Variability in hand contamination based on serial measurements: implications for assessment of hand-cleansing behavior and disease risk. *Am. J. Trop. Med. Hyg.* 84, 510–516.
- Rusin, P., Maxwell, S., Gerba, C., 2002. Comparative surface-to-hand and fingertip-to-mouth transfer efficiency of gram-positive bacteria, gram-negative bacteria, and phage. *J. Appl. Microbiol.* 93, 585–592.
- Schönning, C., Westrell, T., Stenström, T.A., Arnbjerg-Nielsen, K., Hasling, A.B., Højbye, L., Carlsen, A., 2007. Microbial risk assessment of local handling and use of human faeces. *J. Water Health* 5, 117–128.
- Sinclair, R.G., Gerba, C.P., 2011. Microbial contamination in kitchens and bathrooms of rural Cambodian village households. *Lett. Appl. Microbiol.* 52, 144–149.
- Teunis, P.F.M., Reese, H.E., Null, C., Yakubu, H., Moe, C.L., 2016. Quantifying contact with the environment: behaviors of young children in Accra, Ghana. *Am. J. Trop. Med. Hyg.* 94, 920–931.
- U.S. EPA, 2011. *Exposure Factors Handbook 2011 Edition (Final Report)*, 2011. U.S. EPA.
- WHO, 2016. *Quantitative Microbial Risk Assessment: Application for Water Safety Management*. World Health Organization, Geneva, Switzerland.
- Winblad, U., 2004. *Ecological Sanitation (EcoSanRes Programme)*.
- Xue, J., Zartarian, V.G., Özkaynak, H., Dang, W., Glen, G., Smith, L., Stallings, C., 2006. A probabilistic arsenic exposure assessment for children who contact chromated copper arsenate (CCA)-treated playsets and decks, part 2: sensitivity and uncertainty analyses. *Risk Anal.* 26, 533–541.
- Zartarian, V.G., Streicker, J., Rivera, A., Cornejo, C.S., Molina, S., Valadez, O.F., Leckie, J.O., 1995. A pilot study to collect micro-activity data of two- to four-year-old farm labor children in Salinas Valley, California. *J. Expo. Anal. Environ. Epidemiol.* 5, 21–34.